Cytogenetic, Spectral Karyotyping, Fluorescence in Situ Hybridization, and Comparative Genomic Hybridization Characterization of Two New Secondary Leukemia Cell Lines With 5q Deletions, and *MYC* and *MLL* Amplification

Turid Knutsen,[1]* Svetlana Pack,[2] Maria Petropavlovskaja,[1] Hesed Padilla-Nash,[1] Clement Knight,[1] Lyn A. Mickley,[1] Thomas Ried,[1] Patrick C. Elwood,[1] and Susan J. Roberts[1]

[1]Medicine Branch, Genetics Branch, and Cancer Therapeutics Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

[2]Surgical Neurology Branch, National Institute of Neurological Disease and Stroke, National Institutes of Health, Bethesda, Maryland

Cytogenetic studies of patients with therapy-induced acute myeloid leukemia (t-AML) have demonstrated whole chromosome loss or q-arm deletion of chromosomes 5 and/or 7 in a majority of cases. We have established two cell lines, SAML-1 and SAML-2, from two patients who developed t-AML after radiation and chemotherapy for Hodgkin disease. In both cases, the leukemia cells contained 5q deletions. SAML-1 has 58 chromosomes and numerous abnormalities, including der(1)(1qter→1p22::5q31→5qter), der(5)(5pter→5q22::1p22→1pter), +8, der(13)i(13)(q10)del(13)(q11q14.1), and t(10;11). Fluorescence in situ hybridization (FISH) with unique sequence probes for the 5q31 region showed loss of *IL4*, *IL5*, *IRF1*, and *IL3*, and translocation of *IL9*, *DS5S89*, *EGR1*, and *CSFIR* to 1p. SAML-2 has 45 chromosomes, del(5)(q11.2q31) with a t(12;13)ins(12;5), leading to the proximity of *IRF1* and *RB1*, and complex translocations of chromosomes 8 and 11, resulting in amplification of *MYC* and *MLL*. Comparative genomic hybridization and spectral karyotyping were consistent with the G-banding karyotype and FISH analyses. Because a potential tumor suppressor(s) in the 5q31 region has yet to be identified, these cell lines should prove useful in the study of the mechanisms leading to the development of t-AML.

INTRODUCTION

Although chemotherapy has greatly improved the survival for particular types of cancer such as Hodgkin disease (HD), a fraction (2–5%) of these patients will later develop therapy-related myelodysplastic syndrome (t-MDS) and acute myeloid leukemia (t-AML) (Levine and Bloomfield, 1992; Harrison et al., 1998). It is estimated that up to 20% of all cases of AML are therapy related (Kantarjian et al., 1993). Most cases of t-AML are of the M1–M2 and M4–M5 subtype; classification is sometimes difficult, however, because the leukemias frequently demonstrate trilineage involvement and appear to bridge several subtypes (Levine and Bloomfield, 1992). The majority of these cases have specific cytogenetic abnormalities, most commonly the acquired loss of genetic material from chromosomes 5 and 7, either by the development of chromosomal monosomy or as a result of deletion.

Acquired interstitial deletion of 5q is found in a variety of other myeloid disorders, the most frequent being de novo AML and MDS (10–15% of cases) (Nimer and Golde, 1987), including the "5q− syndrome." The hemizygosity resulting from the 5q deletion may unmask a tumor-suppressor gene on the remaining homolog as the result of a mutation of the second allele (Nagarajan, 1996). 5q31 deletion by itself could contribute to the development of the leukemic phenotype if both alleles of the critical gene are required for normal function, as in haploinsufficiency (Schoch et al.,

M. Petropavlovskaja is currently at Montreal General Hospital, Montreal, Quebec, Canada.

C. Knight is currently at Maryland Oncology, P.A., Columbia, MD.

P. Elwood is currently at Hematology-Oncology Department, Licking Memorial Hospital, Newark, OH.

S. Roberts is currently at National Research Council, The National Academies, Washington, DC.

*Correspondence to: Turid Knutsen, Genetics Branch, Center for Cancer Research, National Cancer Institute, NIH, 50 South Drive, Room 1408, Bethesda, MD 20892-8010.
E-mail: knutsent@mail.nih.gov

Received 5 April 2002; Accepted 6 December 2002

DOI 10.1002/gcc.10200

2002). Although the size of the deleted region varies among patients, the consistent loss of 5q31 (Johansson et al., 1993; Le Beau et al., 1993; Willman et al., 1993) suggests that a key regulatory gene resides there. A striking cluster of genes governing normal hemato-lymphoid growth and differentiation, including growth factors, hormone receptors, and proteins involved in signal transduction or transcriptional regulation, have been mapped to the 5q31 region, all residing within a few megabases of each other (Nagarajan, 1996). Although numerous studies have been undertaken by use of a variety of cytogenetic and molecular techniques, an unequivocal critical gene has not been identified.

We have established two new cell lines, designated SAML-1 and SAML-2, from two patients who developed AML secondary to treatment for HD. To our knowledge, this is only the second report of cell lines established from t-AML. The previously described cell line, OHN-GM, was also derived from a patient who developed AML after treatment for HD (Nagai et al., 1997). Both patients in the current study had been treated with radiation and chemotherapy, and developed secondary leukemia 5 to 15 years after initial treatment for HD. Cytogenetic and molecular cytogenetic analyses [G-banding, spectral karyotyping (SKY), fluorescence in situ hybridization (FISH), and comparative genomic hybridization (CGH)] of the cell lines revealed interstitial deletion of 5q and numerous other cytogenetic abnormalities.

MATERIALS AND METHODS

Patient 1 (SAML-1 Cell Line)

This patient was diagnosed with stage IV-B nodular sclerosing Hodgkin disease in the summer of 1976. He was treated with MOPP (mechlorethamine, vincristine, procarbazine, and prednisone) from December 1976 through May 1977. Because of persistent abnormal radiographs and lymphangiograms, chemotherapy was followed by total nodal irradiation from July to December 1977, resulting in a complete remission. Bone marrow studies in 1976 and 1977 showed no evidence of malignant disease. In September 1991, the patient had pancytopenia, including a low platelet count. A bone marrow aspirate contained 67% blasts, leading to a diagnosis of acute myeloid leukemia. The patient received three cycles of idarubicin, cytarabine, and etoposide and went into complete remission, but his leukemia relapsed in June 1992. Reinduction therapy with cytarabine and mitoxantrone in June, and verapamil, idarubicin, cytarabine, and etoposide in August, failed to produce a remission. During September and October, he received 3 weeks of treatment with vincristine, prednisolone, and L-asparaginase, again without response. In November 1992, the bone marrow had 80% myeloblasts, and the patient was transferred to the Fred Hutchinson Cancer Center (Seattle, WA) for a bone marrow transplant. He responded to the induction therapy but died shortly thereafter at age 50.

Patient 2 (SAML-2 Cell Line)

This 36-year-old man became symptomatic in July 1990 and was diagnosed with mixed cellularity Hodgkin disease in March 1991. His initial treatment with MOPP and mantle-field irradiation resulted in complete remission. A relapse 2½ years later, in March 1993, was diagnosed as nodular-sclerosing HD; treatment with EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, and Adriamycin) led to a complete remission. Six months later, however, the patient relapsed with bilateral bone marrow involvement; another round of EPOCH (without Adriamycin) resulted in a partial remission. Recurrent disease from September 1994 through June 1996 was treated with velban, taxol, 9-aminocamptothecan, and vinblastine, with little or no response and with evidence of disease progression. In June 1996, the patient was found to have an elevated white blood cell count, reduced platelets (45,000), and 38% myeloblasts in the bone marrow. He was diagnosed with AML, secondary to treatment for multiply relapsed lymphoma. He died within 2 months of acute respiratory failure, partially because of leukemic sludging in the pulmonary vasculature, at age 42.

Cell Lines

Suspension cultures were established from bone marrow aspirates from patient 1 (specimen obtained within a month of death) and patient 2 (obtained at diagnosis of AML), and designated SAML-1 and SAML-2, respectively. SAML-1 was maintained in RPMI 1640 with 10% fetal bovine serum (FBS), penicillin, streptomycin, and amphotericin B. SAML-2 was maintained in RPMI 1640 with 20% FBS, 10% Giant Cell Tumor Conditioned Medium (IGEN, Gaithersburg, MD), 5% horse serum, and 0.2 ng/ml granulocyte macrophage colony stimulating factor (GM-CSF). Both cell lines were characterized by cytochemical staining of cytospins and fluorescent immunostaining.

Cytogenetic and SKY Analyses

Routine cytogenetic analysis was performed on the cell lines established from the bone marrow cells of the two patients, according to standard techniques. SKY was performed according to the technique of Schröck et al. (1996) and Macville et al. (1997) for identification of all chromosomal abnormalities in the two cell lines. Images were acquired with a spectral cube system (Applied Spectral Imaging, Migdal Haemek, Israel) attached to an epifluorescence microscope (DMRXA, Leica, Wetzlar, Germany), and the emission spectrum was measured with a custom-made triple-band–pass filter (Chroma Technology, Brattleboro, VT). Both probe and DAPI images were acquired.

CGH Analysis

CGH was performed on normal, sex-matched (male) metaphase chromosomes according to a modification of the method described by du Manoir et al. (1993). Average ratio profiles were calculated from 11–15 ratio images per cell line. Ratios of tumor to reference signal of <0.75 and >1.25 were interpreted as representing loss and gain, respectively, of DNA mapped to a particular chromosome region.

FISH Studies

FISH studies were conducted by use of cosmid probes, unique sequence probes for specific human genes, and whole-chromosome and chromosome-arm paints. The following probes were obtained from commercial sources, and hybridizations were performed according to the manufacturers' instructions: Cri du Chat (5p15.2), 5q31, *MYC* (8q24.1), Retinoblastoma (13q14), and *MLL* (11q23) from Oncor (Gaithersburg, MD) or Ventana Medical Systems (Tucson, AZ); *TP53* (SpectrumOrange) from Vysis (Downers Grove, IL); *D5S89* from the American Type Culture Collection (ATCC, Manassas, VA); bacterial artificial chromosome (BAC) probes containing *MDR1* sequences (BAC clone 332G20), and a probe containing sequences 500–1000 kilobases (kb) telomeric to *MDR1* (BAC clone 063L14) from Research Genetics (Huntsville, AL). Additional probes were kindly provided by individual investigators: cosmid probes for *IRF1*, *IL3*, *IL4*, *IL5*, *IL9*, *EGR1*, and *CSFIR* from L. Deaven of the Los Alamos National Laboratory (Los Alamos, NM); *DHFR* from C. Allegra of the National Cancer Institute (NIH, Bethesda, MD); a subtelomeric probe for 5qter from Y. Ning of the National Human Genome Research Institute (NHGRI, NIH, Bethesda, MD); and DNA for chromosome-arm paints 2p, 2q, 20p, and 20q from M. Bittner of NHGRI. The latter probes were combined with whole-chromosome paints 4, 6, 15, and 22, produced in our laboratory, for clarifying results obtained from SKY analysis in SAML-1. Mutation analysis of *TP53* was performed by sequencing of the cDNA corresponding to exons 5–9, the mutated region identified in previous studies. RT-PCR was performed with a 3′ primer corresponding to the sequence 1022–1042 and a 5′ primer corresponding to the sequence 339–359; sequencing was performed by use of nested primers.

RESULTS

Phenotypic Characterization

Phenotypic studies, including cell surface markers, indicated the myeloid origin of both cell lines (Table 1). Wright–Giemsa-stained cells of SAML-1 and SAML-2 were uniformly myeloblastic, with large round nuclei surrounded by a small volume of non-staining cytoplasm. The staining patterns in these two cell lines are consistent with a myelomonocytic leukemia phenotype.

Cytogenetic Analyses (G-Banding and SKY)

The cell line SAML-1 was derived from a bone marrow sample obtained in November 1992. The bone marrow culture was maintained by replacing half of the suspension with fresh medium once a week. It was first submitted for cytogenetic analysis in January 93 (after about eight doublings of the original sample), and subsequent analyses were performed periodically. The possibility of in vitro development of aberrations cannot be discounted; however, the cell line was remarkably stable, and the only differences in the various samplings were the appearance of the der(16)t(16;20) in the February 93 and subsequent harvests, and abnormalities of chromosome 7. All samples had two normal chromosomes 7. A del(7)(q22 or q22q36) appeared in February 93, and the remaining samples all showed a der(7) with multiple copies of the q21q22 bands. The full karyotype is shown in Figure 1A.

Patient 2 had one bone marrow cytogenetic study, which had the following clonal karyotypes: clone 1: 45,XY,del(5),der(8),der(11)x2,der(12)t(12;13)ins(12;5); clone 2 had the same abnormalities plus i(7)(q10). The cell line derived from this marrow sample, SAML-2, revealed the same karyotype as that of clone 1, with the addition of dup(10) and t(Y;15), which apparently developed in vitro. The full karyotype is shown in Figure 2A.

TABLE 1. Comparison of t-AML Cell Lines Secondary to Hodgkin Disease

Characteristic	OHN-GM[a]	SAML-1	SAML-2[b]
Growth requirements	GM-CSF dependent	GM-CSF independent	GM-CSF dependent
MDS phase	Yes (MDS→RAEB→AML	No	No
Time from HD to t-MDS or t-AML	3 years	15 years	5 years
Duration MDS → AML	2 years	No MDS phase	No MDS phase
Survival post AML	4 months	14 months	1 month
Treatment for HD	C-MOPP	MOPP, radiation	MOPP, radiation, EPOCH
Percentage blasts	67%	67%	38%
Cytochemistry	Positive: peroxidase (almost all), nonspecific esterase (10%)	Positive: NAE (96%), CAE (21%)[b] Negative: myeloperoxidase, Sudan Black B	Positive: NAE (33%), CAE (73%) myeloperoxidase (11–15%), Sudan Black B (11–15%)
Cell surface markers	Positive:CD7,CD13,CD33,CD34,CD38, CD71,HLA-DR & c-kit antigens. Negative: Lymphoid antigens, CD41, glycophorin A	Positive: CD13,CD14,CD33. Negative: lymphoid antigens. Pleomorphic blasts	Positive: CD11b, CD13, CD15, CD33, CD14
Ploidy	Hyperdiploid (48)	Hyperdiploid (58)	Hypodiploid (45)
5q abnormality	del(5)(q11.2q31)	der(1)(1qter→1p22::5q31→5qter), der(5)(5pter→5q22::1p22→1pter)	del(5)(q13q31) der(12)t(12;13)ins(12;5)
IRF1	Loss of one allele, no gene rearrangement of 2nd allele	Loss of one allele	Translocation of one allele
7 abnormality	−7	der(7)(?16::7q21::7p13→7q22::7q21→ 7q22::7q21→7q22::1q32→1qter	None
8 abnormality	+8	+8	der(8)t(8;11), der(11)t(8;11)
13 abnormality	+der(13)t(10;13)(q24;q14)	der(13)del(13)(q11q14.1)i(13)(q10) +der(13)(q?) CGH: 13p13→q14 loss, 13q21→qter gain	der(12)t(12;13)(p13.3;q14)ins(12;5)(p13.3; q31q31) CGH:13q11→q14 loss
RB1	Deleted, no RB1 protein	Three copies of *RB1*	Translocation of *RB1* and proximity to *IRF1*
TP53	Two missense point mutations	Normal by FISH and CGH; none of the common mutations	Point mutation; normal by FISH and CGH
Other chromosome abnormalities	+22	t(2;6),t(2;21), t(2;6), dic(1;3), t(10;11), dup(10), t(6;12), del(12), t(4;6), t(16; 20), t(12;17), del(19), t(15;20), t(15;20)	t(Y;15), dup(10)

[a]Nagai et al., 1997.
[b]NAE, alpha-naphthyl acetate esterase; CAE, chloroacetate esterase.

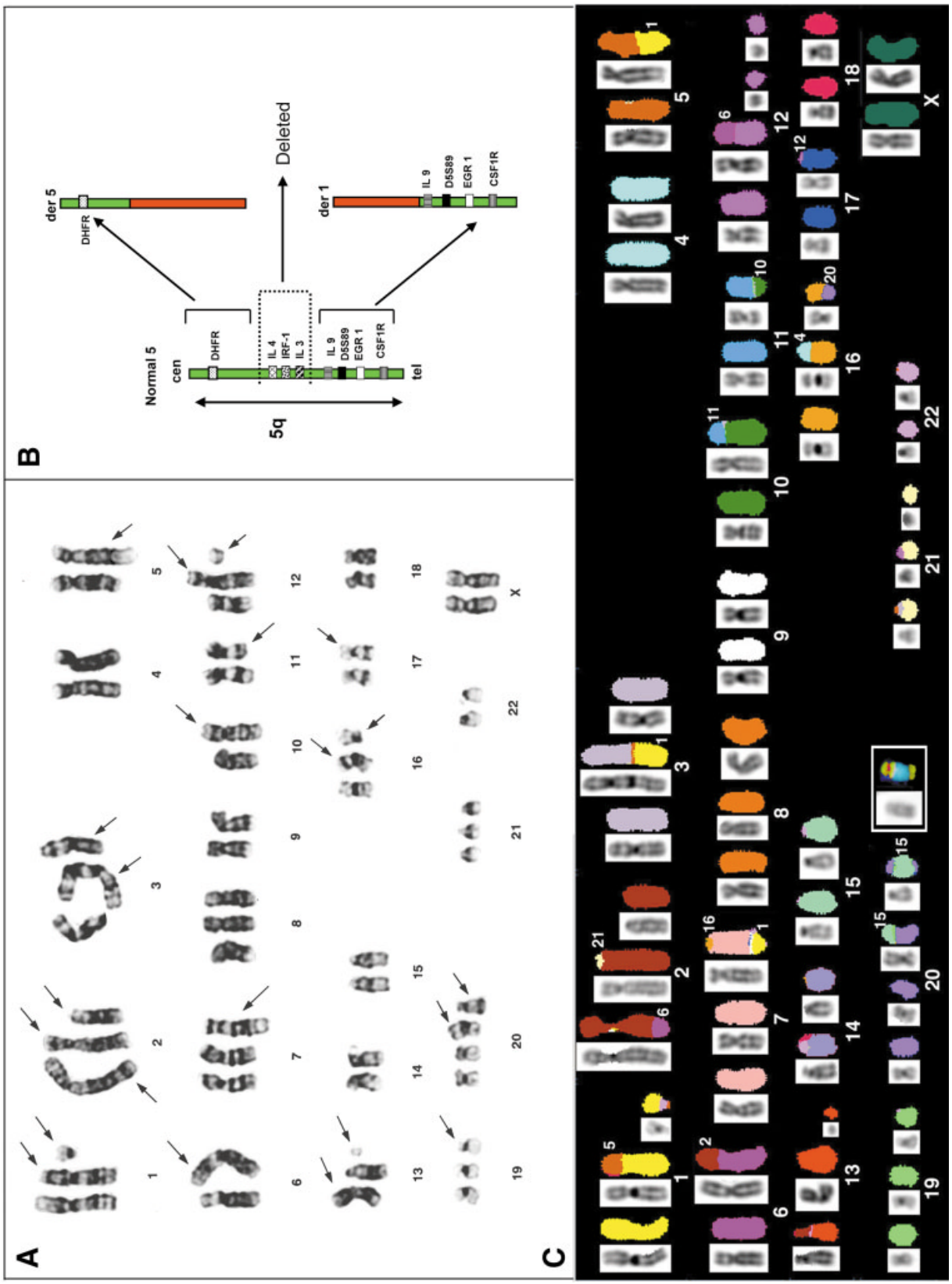
A
B
C
Normal 5
cen
tel
5q
DHFR
IL 4
IRF-1
IL 3
IL 9
D5S89
EGR 1
CSF1R
Deleted
der 5
der 1

Figure I.

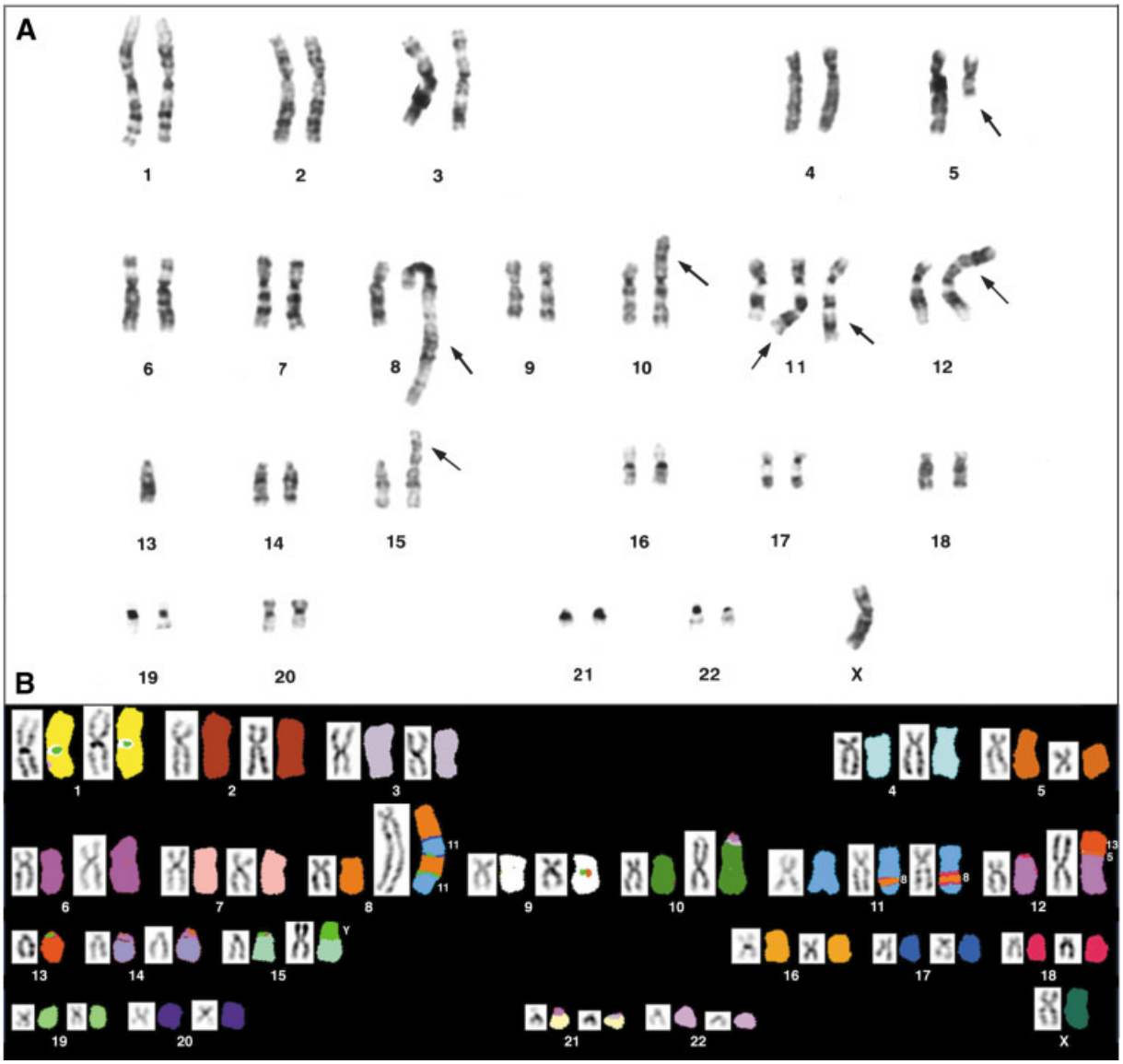

Figure 2. Cytogenetic analysis of cell line SAML-2. **A:** GTG-banded karyotype: 45,X,der(Y;15)(q10;q10),del(5)(q13q31),der(8)(8pter→8qter::11q21→11qter::8q13→8qter::11q21→11qter::11q?23→11q?24),dup(10)(?p15p10),+11,der(11)(11pter→11q21: :8q22.1→8q24.1:: 11q22→11qter)x2,der(12)t(12;13)(p13.3;q14)ins(12;5)(p13.3;q31q31),−13. Arrows indicate structural abnormalities. The karyotype is based on the results of GTG, SKY, CGH, and FISH studies. **B:** SKY karyotype with inverted DAPI and classified chromosome images.

Figure 1. Cytogenetic analysis of cell line SAML-1. **A:** GTG-banded karyotype: 58,X,+X,−Y,der(1)(1qter→1p22::5q31→5qter),+del(1)(pter→p35.3::cen→q11:),der(2)t(2;6)(q33;q23),der(2)t(2;21)(p21;q22),+t(2;6)(p11.1;p21.3),dic(1;3)(q10;q21),+inv(3)(q?q?), der(5)(5pter→5q22::1p22→1pter),+der(7)(?16::7q21→7q21::7p13→7q22::7q21→7q22::7q21→7q22::1q32→1qter),+8, t(10;11)(p12;q14)dup(10)(q25q-ter),der(12)(6pter→6p11.2::12p13→12qter),+del(12)(q11 or q11q24.3),der(13)del(13)(q11q14.1)i(13)(q10), +del(13)(q?),der(16)t(4;16)(?p;p12),+der(16)(16pter→16q11.1::20q11.2→20q13.1),der(17) t(12;17)(?p;p13),+del(19)(p13.2 or p13.2p13.3),+der(20)(15qter→15q22::20p13→20qter),+der(20) (20p11.1→20cen::20q?::20p?::15q22→15q25::20p12→20pter),+21. Arrows indicate structural abnormalities. The karyotype is based on the results of GTG, SKY, CGH, and FISH studies. **B:** Cartoon of chromosome 5 in SAML-1, demonstrating genes retained on chromosome 5, genes lost, and genes translocated to chromosome 1. **C:** SKY karyotype with inverted DAPI and classified chromosome images. Insert in karyotype shows FISH of der(20) with paints for chromosome 15 (blue) and chromosome arms 20p (yellow) and 20q (red) revealing der(20)(20p11.1→20cen::20q?::20p?::15q22→15q25::20p12→20pter).

The complete SKY ideograms and CGH profiles for both cell lines can be viewed at the NCI and NCBI Spectral Karyotyping SKY and Comparative Genomics Hybridization CGH Database (2001) at http://www.ncbi.nlm.nih.gov/sky/skyweb.cgi.

FISH Studies

In SAML-1, FISH studies with a 5q31 probe (Oncor) showed translocation of this region to 1p, and FISH with probes for genes localized to the 5q region revealed the following: DHFR was retained on chromosome 5; *IL4*, *IRF1*, and *IL3* were de-

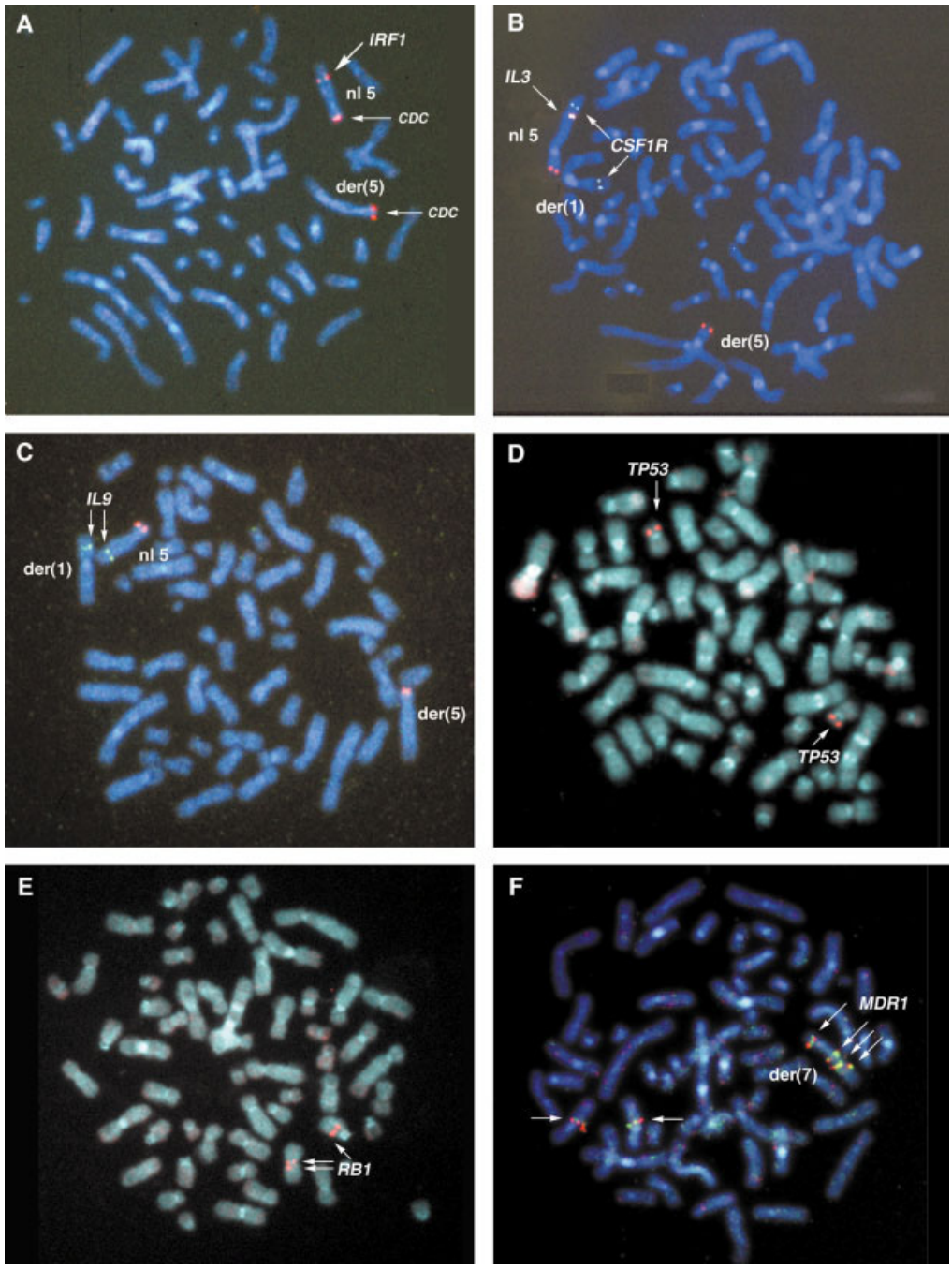

Figure 3. FISH studies in SAML-1. **A:** Deletion of *IRF1* in der(5); CDC marks 5p in both the normal chromosome 5 and der(5). **B:** Deletion of *IL3* in der(5) and translocation of *CSFIR* to der(1); CDC marks 5p in both the normal chromosome 5 and der(5). **C:** Translocation of *IL9* from chromosome 5 to der(1); CDC marks 5p in both the normal chromosome 5 and der(5). **D:** *TP53* in two chromosomes 17, one of which is a der(17)t(12;17)(?p;p13). **E:** Single *RB1* in a normal chromosome 13 and two copies of *RB1* in the der(13)del(13)i(13) (arrows). **F:** FISH with BAC-derived probes for *MDR1* (green) and sequences just telomeric to it (red): two normal chromosomes 7 with single copies of each probe (single arrows) and der(7) with four copies of each probe (arrows).

leted; and *IL9*, *D5S89*, *EGR1*, and *CSFIR* were translocated to 1p (Figs. 1B and 3A–C). Although SAML-1 had a translocation involving 17p, der(17)t(12;17)(?p;p13), CGH showed a normal profile for chromosome 17, FISH showed two copies of the *TP53* gene (Fig. 3D), and sequence analysis revealed no mutations in exons 5–9, the region where mutations of *TP53* were previously described (data not shown). This cell line had three copies of *RB1* (located at 13q14.2) as a result of the

formation of an isochromosome 13 (Fig. 3E). SAML-1 showed amplification of the *MDR1* gene, located at 7q21, both by FISH and CGH analysis in an abnormal chromosome 7 with multiple copies of 7q21–q22. FISH analysis with two BAC-derived probes, one containing *MDR1* sequences and the other containing sequences 500–1000 kb telomeric to *MDR1*, indicates that the *MDR1* gene remained intact (Fig. 3F) because *MDR1* transcribes in a centromeric direction. Whereas FISH and CGH indicated amplification of the gene, no overexpression or gene rearrangements were detected by RNase protection assay (data not shown).

In SAML-2, band 5q31 was split, with a portion remaining on the deleted 5q and another portion translocated to the abnormal chromosome der(12)t(12;13)ins(12;5) (Fig. 4A, B). *IRF1* was part of the 5q31 band translocated to the der(12) (Fig. 4A); this rearrangement also resulted in the translocation of the *RB1* gene, placing it near the *IRF1* gene on the der(12) (Fig. 4A). FISH with the 5qter subtelomeric probe gave signals at the terminal position on both the normal and deleted chromosomes, confirming that the 5q deletion in SAML-2 was interstitial. SKY studies of SAML-2 also revealed two derivative chromosomes involving exchange and duplication of material between chromosomes 8 and 11 (Fig. 2), and FISH studies revealed translocation and amplifications of the oncogenes *MYC* (8q24.1) and *MLL* (11q23) (Fig. 4C, D). It is not clear that the rearrangements involved breaks in either *MYC* or *MLL*, but northern analysis of the *MYC* mRNA demonstrated that the transcript was overexpressed but of germline size. Cytogenetic and CGH studies of SAML-2 demonstrated no abnormalities of chromosome 17, and FISH studies revealed two copies of the *TP53* gene at 17p13.1 (Fig. 4E). However, Castro et al. (2000) found loss of heterozygosity for 17p in SAML-2, indicating deletion of one allele of *TP53*. Sequencing of the remaining allele showed that it contained the common *TP53* mutation R273H, with no normal copies of *TP53* in this cell line.

CGH Studies

The CGH profiles for SAML-1 and SAML-2 are shown in Figure 5A and B, respectively. Both cell lines showed partial loss of 5q by CGH, 5q21–q31 in SAML-1 and 5q13–q31 in SAML-2. Loss of 13q11–14 was also noted in both cell lines: in SAML-1, it was the result of the formation of der(13)del(13)(q11q14.1)i(13)(q10), and in SAML-2, as a result of a translocation, t(12;13)ins(12;5). In both cell lines, *RB1*, located in the middle of band 13q14, was retained, and SAML-1 contained three copies as a result of the formation of the der(13)del(13)i(13).

DISCUSSION

Of t-AML cases, 70–95% exhibit clonal cytogenetic abnormalities, an incidence higher than that in de novo AML. The karyotypic pattern of t-AML strongly resembles that of adult de novo AML (Heim and Mitelman, 1995), but the incidence of abnormalities of chromosomes 5 and 7 is much higher in t-AML (Le Beau et al., 1986). Other chromosomal abnormalities more commonly reported in t-AML than in de novo AML include +8 or +21, or abnormalities of 6p, 11q, and 12p. In both t-AML and de novo AML, +8 and −17 are among the most frequent abnormalities (Iurlo et al., 1989; Johansson et al., 1991). SAML-1 exhibited structural abnormalities in many of these same chromosomes (3, 6, 8, 11, 12, 17, and 21) (Table 1 and Fig. 1).

OHN-GM is the only other therapy-related leukemia cell line that has been reported (Nagai et al., 1997). Several features of this cell line are similar to the SAML-1 and SAML-2 cell lines, as summarized in Table 1 and described below. Deletion of *TP53* has been reported in a number of t-AML and t-MDS patients and has been associated with 5q loss or deletion, a complex karyotype, and poor prognosis (Merlat et al., 1999; Christiansen et al., 2001). Although the three t-AML cell lines did not show cytogenetic abnormalities involving *TP53*, both OHN-GM and SAML-2 had *TP53* mutations with no normal alleles (Table 1). Castro et al. (2000) demonstrated a correlation between *TP53* mutations and 5q13 deletion, which is consistent with *TP53* mutations in OHN-GM and SAML-2, both of which have 5q13 deletions, but only wild-type *TP53* in SAML-2, which has deletion 5q21q31. Cytogenetically normal chromosomes 17 with two copies of *TP53* by FISH in cases with only mutated alleles of *TP53*, as was observed in OHN-GM and SAML-2, can be explained by the loss of the normal chromosome with reduplication of the chromosome containing the mutated allele (Christiansen et al., 2001).

OHN-GM contains a deletion of the *RB1* gene on 13q as a result of the 10;13 translocation and lacks RB1 protein. SAML-1 has three copies of *RB1* (Fig. 3E) and SAML-2 has a translocation of the *RB1* gene to the der(12)t(12;13)ins(12;5), placing it in proximity to *IRF1* in the 5q31.1 insertion (Fig. 4A). Translocations between 12p11.2–13 and

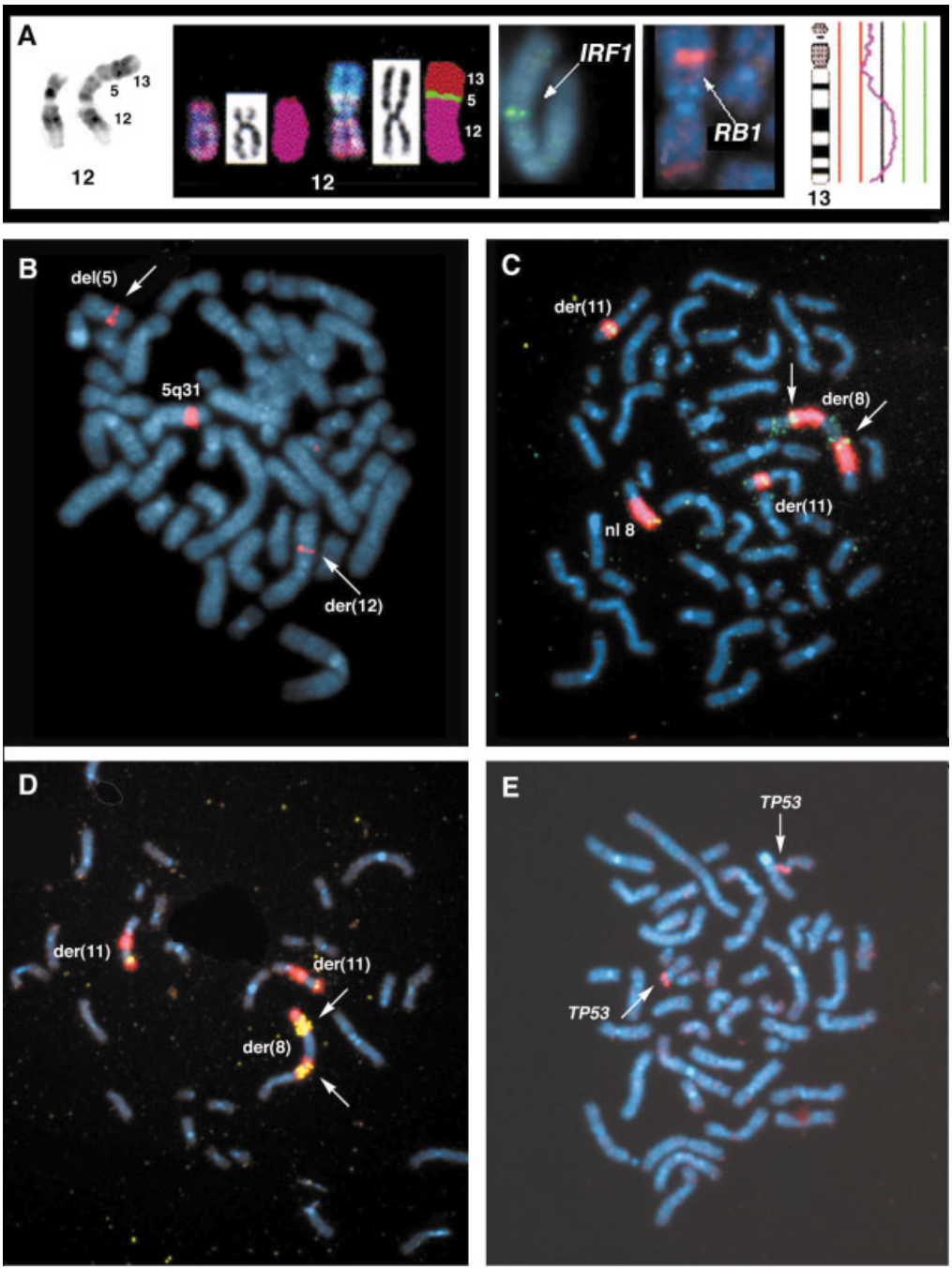

Figure 4. FISH studies in SAML-2. **A:** GTG-banding, SKY, and FISH studies of the der(12)t(12;13)(p13.3;q14)ins(12;5)(p13.3;q31q31), showing translocation and proximity of the *IRF1* (from 5q31.1) and *RB1* genes (from 13q14.2) to the der(12), and loss of 13q11→q14, as shown by CGH. **B:** Split of band 5q31 (red) between the del(5) and the der(12). **C:** Paint probe for 8q (red) and an *MYC* probe (yellow): *MYC* is present in single copies in the single normal 8, in the portion of 8q inserted into each of the two copies of der(11), and in both portions of 8q involved in the der(8) (arrows). **D:** Paint probe for 11q (red) and an *MLL* probe (yellow): *MLL* is present in single copies in the normal 11 (not shown) and in the der(11) (two copies), and amplified in both 11q portions of the der(8) (arrows). **E:** *TP53* (arrows) in two normal-appearing chromosomes 17 (no normal allele for *TP53* was detected by sequencing).

13q11–14 are rare, but non-random events in acute leukemia (Chase et al., 1999). Studies by Coignet et al. (1999) in patients with a t(12;13)(p12;q14) revealed the presence of a myeloid-specific breakpoint cluster region with the breakpoint in 13q14 centromeric to *RB1*, as was the case in SAML-2.

IRF1, which is frequently but not invariably lost in 5q31 deletions (Boultwood et al., 1993; Willman

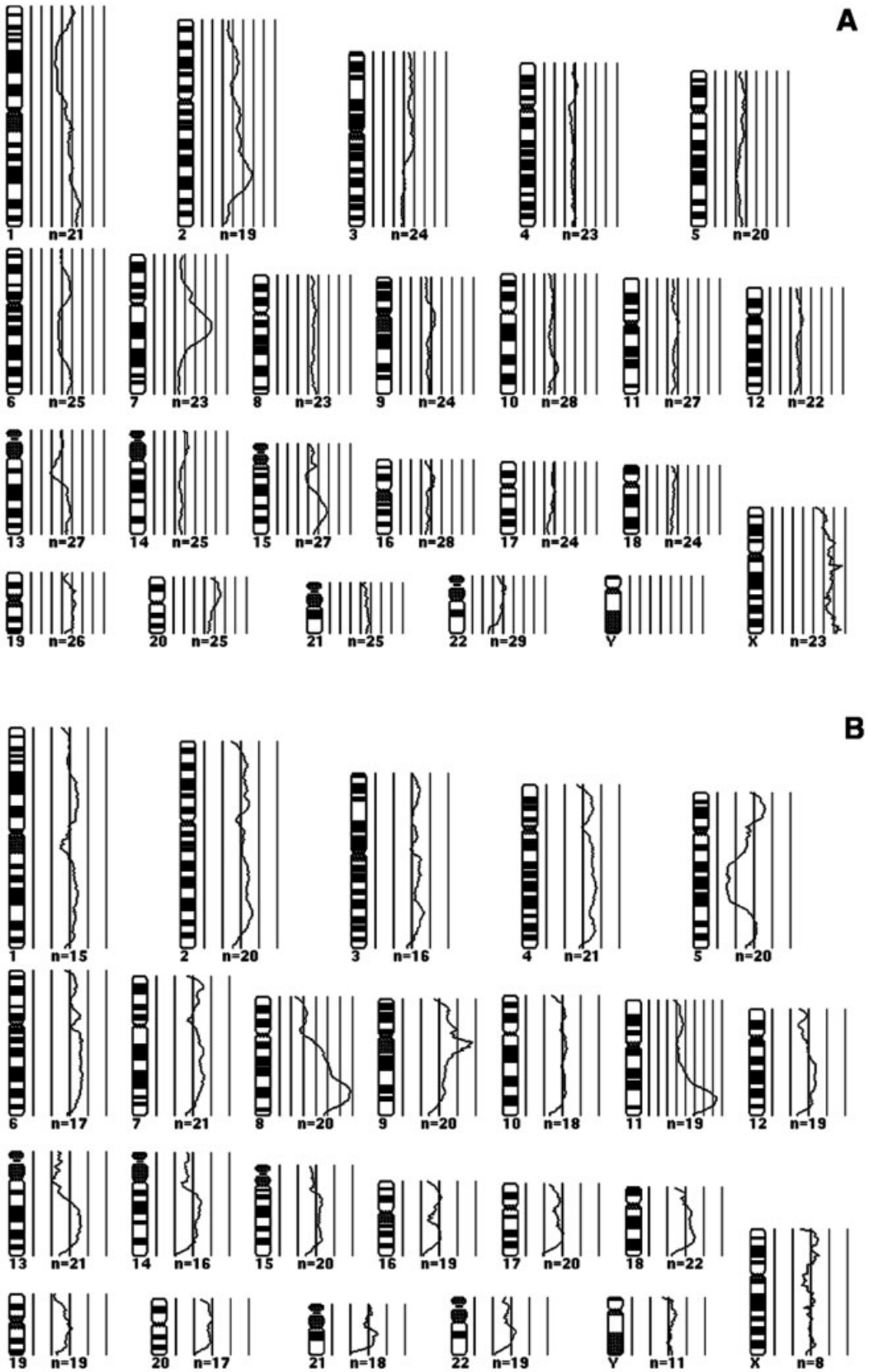

Figure 5. CGH profiles. **A:** SAML-1, demonstrating gains at 1p35.3→pter, 1q, 2q11.1→q33 (peak 2q24→q33), 3pter→q21, 6p10→p21.3, 6q23→qter, 7p13→q22 (peak 7q11.2→q22), 8 (probable), 13q21→qter, 15q21→qter (peak 15q22→q25), 19p13.2→qter, 20 (peak 20p12→q11.1), and 21, and losses at 5q21→q31, 13q12→q14, and 22q13.3. **B:** SAML-2, demonstrating gains at 8q11.1→qter (peak 8q22.1→q24.1) and 11 (peak 11q21→qter), and losses at 5q13→q31 and 13q11→q14; the regions of chromosomes 8 and 11 that were amplified were those containing the oncogenes *MYC* (8q24.1) and *MLL* (11q23).

et al., 1993), has received much attention because it is a transcription factor that regulates expression of genes governing the differentiation, growth suppression, and apoptosis (both *TP53*-dependent and -independent) of normal hematopoietic cells (Green et al., 1999). All three t-AML cell lines had rearrangements and deletions in the vicinity of *IRF1*: SAML-1 and the OHN-GM line (Nagai et al., 1997) had loss of one allele of *IRF1*, and *IRF1* was translocated to the der(12) in SAML-2 (Fig. 4A).

Both SAML-1 and SAML-2 have cytogenetic abnormalities involving chromosome 8 and 11q. SAML-1 has +8 and t(10;11)(p12;q14.2). The latter is an infrequent, but recurring abnormality in M4 and M5 AML and T-ALL (GFCH, 1991; Carlson et al., 2000). SAML-2 has two derivative chromosomes involving exchange and duplication of material between chromosomes 8 and 11, with amplification of *MYC* and *MLL*. There appear to be two different types of 11q23/*MLL* involvement in AML and MDS. The most frequent type exhibits balanced translocation of 11q23, *MLL* rearrangement, and relatively simple karyotypes. The rearrangement is frequent in myeloid, lymphoid, and biphenotypic leukemia, indicating possible involvement of a gene affecting differentiation and proliferation (Rowley, 1993). Therapy-related leukemias with this rearrangement are associated with chemotherapy by use of drugs that target DNA-topoisomerase (topo II) (Felix et al., 1993; Super et al., 1993). Some 11q23 patients exhibit intrachromosomal amplification of unrearranged *MLL*, deletion of 5q, complex karyotypes, and mutation of *TP53* (Felix et al., 1998; Andersen et al., 2001). A few reported cases have unbalanced translocations of 11q/*MLL* similar to those described here for SAML-2. A megakaryoblast cell line, UoC-M1, established from a patient with M1 AML and del(5)(q13q34), had alternating segments of chromosomes 9 and 11 and four copies of the *MLL* gene (Allen et al., 1998), and a case of de novo AML with del(5q) showed alternating insertions involving chromosomes 11 and 15 and multiple copies of *MLL* (Struebel et al., 2000).

In this study of two new secondary leukemia cell lines, we used a variety of cytogenetic and molecular cytogenetic techniques to demonstrate numerous chromosomal abnormalities typical of t-AML, including those associated with alkylating agents and topoisomerase-II inhibitors. Because the tumor-suppressor gene(s) in the 5q31 critical region has yet to be positively identified despite intensive efforts, these cell lines, both of which had involvement of 5q31, should prove useful in studies of the cellular and genetic basis of therapy-induced AML. Cell line SAML-1 has been submitted to ATCC and will be available contingent on successful testing by ATCC; it was deposited as ATCC CRL-2776.

ACKNOWLEDGMENTS

We extend sincere thanks to Robert Robey and Dr. Michael Difilippantonio for assistance with the figures; to Jane Trepel for immunocytochemistry studies; to Drs. Lawrence Deaven, Carmen Allegra, Yi Ning, and Michael Bittner for providing probes for FISH studies; to Roy Knutsen for editorial assistance; and to Dr. Tito Fojo for helpful discussions.

REFERENCES

Allen RJ, Smith SD, Moldwin RL, Lu M-M, Giordano L, Vignon C, Suto Y, Harden A, Tomek R, Veldman T, Ried T, Larson RA, Le Beau MM, Rowley JD, Zeleznik-Le N. 1998. Establishment and characterization of a megakaryoblast cell line with amplification of *MLL*. Leukemia 12:1119–1127.

Andersen MK, Christiansen DH, Kirchhoff M, Pedersen-Bjergaard J. 2001. Duplication or amplification of chromosome band 11q23, including the unrearranged *MLL* gene, is a recurrent abnormality in therapy-related MDS and AML, and is closely related to mutation of the *TP53* gene and to previous therapy with alkylating agents. Genes Chromosomes Cancer 31:33–41.

Boultwood J, Fidler C, Lewis S, MacCarthy A, Sheridan H, Kelly S, Oscier D, Buckle VJ, Wainscoat JS. 1993. Allelic loss of IRF1 in myelodysplasia and acute myeloid leukemia: retention of IRF1 on the 5q− chromosome in some patients with the 5q− syndrome. Blood 82:2611–2616.

Carlson KM, Vignon C, Bohlander S, Martinez-Climent JA, Le Beau MM, Rowley JD. 2000. Identification and molecular characterization of *CALM/AF10* fusion products in T cell acute lymphoblastic leukemia and acute myeloid leukemia. Leukemia 14:100–104.

Castro PD, Liang JC, Nagarajan L. 2000. Deletions of chromosome 5q13.3 and 17p loci cooperate in myeloid neoplasms. Blood 95: 2138–2143.

Chase A, Reiter A, Burci L, Cazzaniga G, Biondi A, Pickard J, Roberts IAG, Goldman JM, Cross NCP. 1999. Fusion of ETV6 to the caudal-related homeobox gene CDX2 in acute myeloid leukemia with the t(12;13)(p13;q12). Blood 93:1025–1031.

Christiansen DH, Andersen MK, Pedersen-Bjergaard J. 2001. Mutations with loss of heterozygosity of *p53* are common in therapy-related myelodysplasia and acute myeloid leukemia after exposure to alkylating agents and significantly associated with deletion or loss of 5q, a complex karyotype, and a poor prognosis. J Clin Oncol 19:1405–1413.

Coignet LJA, Lima CSP, Min T, Streubel B, Swansbury J, Telford N, Swanton S, Bowen A, Nagai M, Catovsky D, Fonatsch C, Dyer MJS. 1999. Myeloid- and lymphoid-specific breakpoint cluster regions in chromosome band 13q14 in acute leukemia. Genes Chromosomes Cancer 25:222–229.

du Manoir S, Speicher MR, Joos S, Schröck E, Popp S, Döhner H, Kovacs G, Robert-Nicoud M, Lichter P, Cremer T. 1993. Detection of complete and partial chromosome gains and losses by comparative genomic in situ hybridization. Hum Genet 90:590–610.

Felix CA, Winick NJ, Negrini M, Bowman WP, Croce CM, Lange BJ. 1993. Common region of *ALL-1* gene disrupted in epipodophyllotoxin-related secondary acute myeloid leukemia. Cancer Res 53:2954–2956.

Felix CA, Megonigal MD, Chervinsky DS, Leonard DGB, Tsuchida N, Kakati S, Block AMW, Fisher J, Grossi M, Salhany KI, Jani-Sait N, Aplan PD. 1998. Association of germline p53 mutation with *MLL* segmental jumping translocation in treatment-related leukemia. Blood 91:4451–4456.

Green WB, Slovak ML, Chen I-M, Pallavicini M, Hecht JL, Willman CL. 1999. Lack of IRF-1 expression in acute promyelocytic

leukemia and in a subset of acute myeloid leukemias with del(5)(q31). Leukemia 13:1960–1971.
Groupe Français de Cytogénétique Hématologique (GFCH). 1991. t(10;11)(p13–14;q14–21): a recurrent translocation in T-cell acute lymphoblastic leukemia. Genes Chromosomes Cancer 3:411–415.
Harrison CN, Vaughan Hudson G, Devereux S, Linch DC. 1998. Outcome of secondary myeloid malignancy in Hodgkin's disease: the BNLI experience. Eur J Haematol 61:109–112.
Heim S, Mitelman F. 1995. Cancer cytogenetics, Second Ed. New York: Wiley-Liss.
Iurlo A, Mecucci C, Van Orshoven A, Michaux J-L, Boogaerts M, Noens L, Bosly A, Louwagie A, Van Den Berghe H. 1989. Cytogenetic and clinical investigations in 76 cases with therapy-related leukemia and myelodysplastic syndrome. Cancer Genet Cytogenet 43:227–241.
Johansson B, Mertens F, Heim S, Kristoffersson U, Mitelman F. 1991. Cytogenetics of secondary myelodysplasia (sMDS) and acute nonlymphocytic leukemia (sANLL). Eur J Haematol 47: 17–27.
Johansson B, Mertens F, Mitelman F. 1993. Cytogenetic deletion maps of hematological neoplasms: circumstantial evidence for tumor suppressor loci. Genes Chromosomes Cancer 8:205–218.
Kantarjian HM, Estey EH, Keating MJ. 1993. Treatment of therapy-related leukemia and myelodysplastic syndrome. Hematol Oncol Clin North Am 7:81–107.
Le Beau MM, Albain KS, Larson RA, Vardiman JW, Davis EM, Blough RR, Golomb HM, Rowley JD. 1986. Clinical and cytogenetic correlations in 63 patients with therapy-related myelodysplastic syndromes and acute nonlymphocytic leukemia: further evidence for characteristic abnormalities of chromosomes no. 5 and 7. J Clin Oncol 4:325–345.
Le Beau MM, Espinosa R III, Neuman WL, Stock W, Roulston D, Larson RA, Keinanen M, Westbrook CA. 1993. Cytogenetic and molecular delineation of the smallest commonly deleted region of chromosome 5 in malignant myeloid diseases. Proc Natl Acad Sci USA 90:5484–5488.
Levine EG, Bloomfield CD. 1992. Leukemias and myelodysplastic syndromes secondary to drug, radiation, and environmental exposure. Semin Oncol 19:47–84.
Macville M, Veldman T, Padilla-Nash H, Wangsa D, O'Brien P, Schröck E, Ried T. 1997. Spectral karyotyping, a 24-colour FISH technique for the identification of chromosomal rearrangements. Histochem Cell Biol 108:299–305.
Merlat A, Lai JL, Sterkers Y, Demory JL, Bauters F, Preudhomme C, Fenaux P. 1999. Therapy-related myelodysplastic syndrome and acute myeloid leukemia with 17p deletion. A report on 25 cases. Leukemia 13:250–257.
Nagai M, Fujita M, Ikeda T, Ohmori M, Kuwabara H, Yamaoka G, Tanaka K, Kamada N, Taniwaki M, Inoue T, Irino S, Takahara J. 1997. Alterations of p53 and Rb genes in a novel human GM-CSF-dependent myeloid cell line (OHN-GM) established from therapy-related leukaemia. Br J Haematol 98:392–398.
Nagarajan L. 1996. Deletion of chromosome 5 (5q−) in leukemia: current knowledge. In: Freireich EG, Kantarjian H, editors. Molecular genetics and therapy of leukemia. Boston: Kluwer Academic. p 19–29.
Nimer SD, Golde DW. 1987. The 5q− abnormality. Blood 70:1705–1712.
Rowley JD. 1993. Rearrangements involving chromosome band 11q23 in acute leukaemia. Cancer Biol 4:377–385.
Schoch C, Haferlach T, Bursch S, Gerstner D, Schnittger S, Dugas M, Kern W, Löffler H, Hiddemann W. 2002. Loss of genetic material is more common than gain in acute myeloid leukemia with complex aberrant karyotype: a detailed analysis of 125 cases using conventional chromosome analysis and fluorescence in situ hybridization including 24-color FISH. Genes Chromosomes Cancer 35:20–29.
Schröck E, du Manoir S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA, Ning Y, Ledbetter DH, Bar-Am I, Soenksen D, Garini Y, Ried T. 1996. Multicolor spectral karyotyping of human chromosomes. Science 273:494–497.
Struebel B, Valent P, Jäger U, Edelhäuser M, Wandt H, Wagner T, Büchner T, Lechner K, Fonatsch C. 2000. Amplification of the *MLL* gene on double minutes, a homogeneously staining region, and ring chromosomes in five patients with acute myeloid leukemia or myelodysplastic syndrome. Genes Chromosomes Cancer 27:380–386.
Super HJG, McCabe NR, Thirman MJ, Larson RA, Le Beau MM, Pedersen-Bjergaard J, Philip P, Diaz MO, Rowley JD. 1993. Rearrangements of the *MLL* gene in therapy-related acute myeloid leukemia in patients previously treated with agents targeting DNA-topoisomerase II. Blood 82:3705–3711.
Willman CL, Sever CE, Pallavicini MG, Harada H, Tanaka N, Slovak ML, Yamamoto H, Harada K, Meeker TC, List AF, Taniguchi T. 1993. Deletion of *IRF-1*, mapping to chromosome 5q31.1, in human leukemia and preleukemic myelodysplasia. Science 259:968–971.